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Molecular phylogeny reveals *Megacollybia virosa* is a *Cantharocybe*

T.K. Arun Kumar[1*] & P. Manimohan[2]

[1]*Department of Botany, The Zamorin's Guruvayurappan College, Kerala, 673014, India*
[2]*Department of Botany, University of Calicut, Kerala 673635, India*
* Correspondence to: *tkakumar@gmail.com*

Abstract — Nuclear LSU rDNA sequence analysis unequivocally places an agaric currently designated as *Megacollybia virosa* in *Cantharocybe*. Based on this molecular evidence, *Cantharocybe virosa* comb. nov. is proposed. This transfer results in the recognition of a third distinct species of *Cantharocybe*. The collections from India form the first record of the genus outside the North American continent. Phenetically, *C. virosa* shares with the type species of the genus, *C. gruberi*, the clitocyboid habit, distinct cheilocystidia, convex pileus with an inrolled margin, subdecurrent to decurrent lamellae, thickset stipe, ellipsoid, thin-walled, hyaline, smooth and inamyloid basidiospores, clamped hyphae, subregular to regular lamellar trama, a cutis-type pileipellis disrupted to form trichodermal patches, pileipellis hyphae with plasmatic pigment, and cystidioid terminal cells. A key to the known species is provided.

Key words — *Agaricales*, *Basidiomycota*, systematics, taxonomy

Introduction

Cantharocybe is a little studied agaric genus (*Agaricales, Basidiomycota*) so far known only from North and Central America. The genus was described by Bigelow & Smith (1973) to accommodate a clitocyboid agaric with robust, yellowish basidiomata, lecythiform or mucronate cheilocystidia, oblong or subcylindric, smooth, inamyloid basidiospores, and clamped hyphae. The genus was originally typified by *Clitocybe gruberi* A.H. Sm. (Smith 1944) from Idaho based on a single sporocarp but subsequently collected from New Mexico, California, and Washington as well. According to Bigelow & Smith (1973), the combination of yellow pigmentation, the unusual cheilocystidia, and subcylindric basidiospores of this species warranted its transfer from *Clitocybe* to a new genus. *Cantharocybe* remained a monotypic genus for nearly three decades, but very recently, another species, *C. brunneovelutina* Lodge et al., was described from Belize (Ovrebo et al. 2011). Ovrebo et al. (2011)

emended the genus concept by widening the range of states of characters such as the texture and color of pileus surface and shape of both basidiospores and cheilocystidia. Although the distinctiveness of *Cantharocybe* as a genus is supported by molecular analyses within the *Agaricales*, the family within which *Cantharocybe* should be placed is uncertain (Binder et al. 2010, Ovrebo et al. 2011). *Cantharocybe* is currently placed among the basal genera of the hygrophoroid clade (i.e., *Hygrophoraceae*) based on molecular analyses with low support (Binder et al. 2010; Ovrebo et al. 2011).

Repeated collections of an agaric from various parts of Kerala State, India have been made in the last two decades. As these particular collections defied identification even to the genus level, it remained unpublished for quite a long time. Eventually it was described as *Megacollybia virosa* based solely on morphology (Manimohan et al. 2010). Lingering doubts about its identity prompted us to undertake a molecular analysis, which revealed that it is closely allied to the two species of *Cantharocybe* mentioned above. The results of our molecular analysis are presented and discussed here.

Materials & methods

DNA was extracted from authentic material (TENN063483) of *Megacollybia virosa* deposited at the Herbarium of the University of Tennessee. Detailed voucher information is available in Manimohan et al. (2010). DNA was extracted from dried tissue using the procedure described in Mata et al. (2007). The nuclear large subunit (LSU) ribosomal RNA region was amplified and sequenced with primers LROR and LR5. Sequences were edited in Sequencher 4.10.1 (Gene Codes Corp., Ann Arbor, Michigan), the segments with ambiguous bases trimmed from the ends, and similar sequences were searched in GenBank with the BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) algorithm. Initial BLAST queries showed close affinities with *Cantharocybe brunneovelutina* (92% sequence identity) and *C. gruberi* (91% sequence identity). An LSU sequence dataset was assembled with sequences of taxa that showed >90% similarities with the newly generated sequence, additional sequences of representative members of genera (including outgroup *Phyllotopsis nidulans* (Pers.) Singer) selected based on Ovrebo et al. (2011), and sequences of representative species of *Megacollybia* (Hughes et al. 2007) were downloaded from GenBank and assembled into a dataset. The newly generated sequence was deposited in GenBank with accession number JX101471.

The LSU sequence dataset was manually aligned in CLUSTALX 2.1 and then examined in Se-Al 2.0a11 (Andrew Rambaut, University of Edinburgh, U.K. http://tree.bio.ed.ac.uk/software/seal). The region analyzed contained a total of 1452 bp. The alignment was deposited in TreeBase with submission number 12767 (http://purl.org/phylo/treebase/phylows/study/TB2:S12767). Maximum Parsimony (MP) analysis was conducted in PAUP* 4.0b10 (Swofford 2002) using a heuristic search option with TBR branch swapping, 1000 replicates using random step-wise addition, and holding one tree at each step. All minimal length trees were saved, and maxtrees was set as unrestricted. Bootstrap analysis (Felsenstein 1985) was carried out to evaluate support for

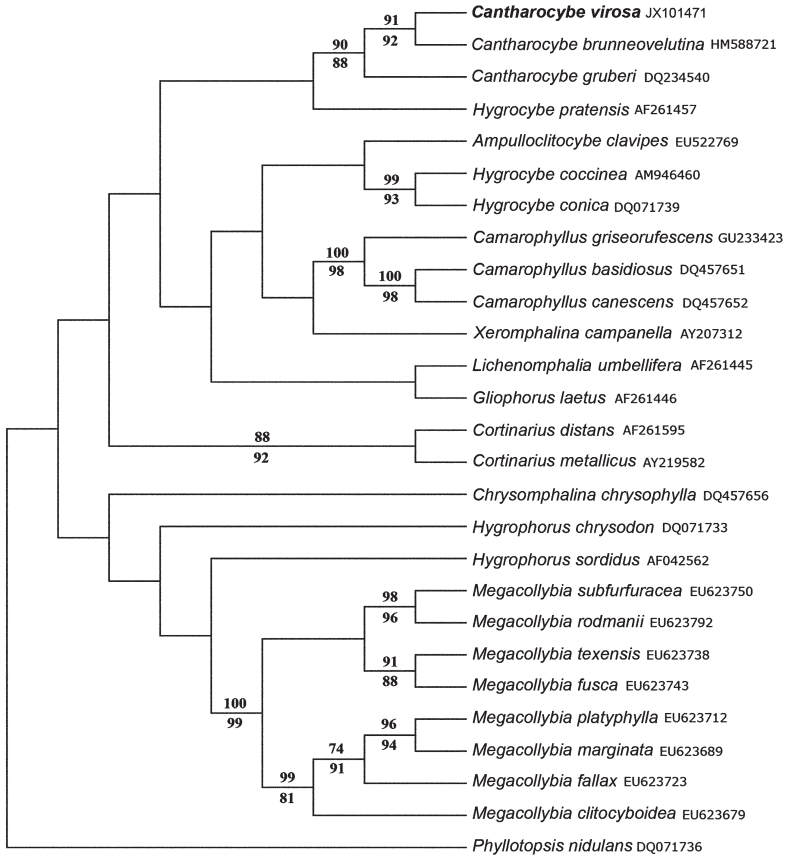

Figure 1. One of four equally parsimonious trees generated during the phylogenetic analyses of LSU rDNA sequences. Numbers above branches indicate MP bootstrap values and numbers below branches indicate BI clade credibility (posterior probability) values. Only values greater than 70% are shown. GenBank accession numbers are provided.

the branching topologies with a full heuristic search option on 1000 replicates. Bayesian inference (BI) analysis was done using MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003). The general time reversible model with gamma distributed rate variation option was selected. Multiple independent analyses were done for 1 million generations with trees saved every 100 generations. Options for incremental heating scheme, chain number, and priors were set to MrBayes default settings. The first 1000 samples were burned before calculating posterior probabilities.

Manimohan et al. (2010) is followed for the morphological description. Color codes refer to Kornerup & Wanscher (1978).

Results

Megacollybia virosa is resolved in a clade with *C. brunneovelutina* and *C. gruberi* with strong branch support in both Bayesian and parsimony analyses (Fig. 1). The analysis also places *Hygrocybe pratensis* (Fr.) Murrill (≡ *Camarophyllus pratensis* (Fr.) P. Kumm.) as the sister group to *Cantharocybe* but with no support. Three species of *Camarophyllus* (= *Cuphophyllus*) were included in the analysis group with species of *Xeromphalina*, *Ampulloclitocybe,* and *Hygrocybe*. Although a relationship of these taxa with *Cantharocybe* is indicated, the placement is not well supported. Eight representative species of the genus *Megacollybia* form a distinct clade with strong support in all the maximum parsimony and maximum likelihood analyses. *Cantharocybe virosa* shows no close phylogenetic affinities with members of *Megacollybia*.

Discussion

DNA sequencing and analysis of the nLSU gene region supports a close phylogenetic relationship between *Megacollybia virosa* and the two known species of *Cantharocybe.* Although initially described as a species of *Megacollybia*, the distinctiveness of *M. virosa* had been recognized based on its abundant cheilocystidia of a unique morphology and the distinctive terminal elements of the pileipellis (Manimohan et al. 2010). The cheilocystidia in *M. virosa* feature a clavate, cylindrico-clavate, or ventricose basal part and an upper part extending into an elongated neck with or without a rounded capitulum (Fig. 2b). While this unique cystidial morphology is not characteristic of *Megacollybia*, it is seen in the type species of *Cantharocybe, C. gruberi*. Phenetically, apart from the morphology of cheilocystidia, *M. virosa* shares the following characters with *Cantharocybe*: robust clitocyboid basidiomata (Fig. 2a), convex pileus with an inrolled margin, subdecurrent to decurrent lamellae, thickset stipe, ellipsoid, thin-walled, hyaline, smooth and inamyloid basidiospores (Fig. 2c), clamped hyphae, subregular to regular lamellar trama, a cutis-type pileipellis disrupted to form trichodermal patches, and pileipellis hyphae with plasmatic pigment and cystidioid terminal cells. At the same time, it differs from both *C. gruberi* and *C. brunneovelutina* in having a grayish brown pileus surface. Additionally, *C. gruberi* differs in having much larger, narrowly elliptic to oblong basidiospores, whereas *C. brunneovelutina* has a unique cystidial morphology with a resemblance to basidia. According to Matheny et al. (2006), *C. gruberi* belongs to pluteoid clade and in Moncalvo et al.'s (2002) analysis, the relationships of *C. gruberi* remained unresolved although it formed a distinct clade with *Camarophyllus pratensis*. The placement of *Camarophyllus* (= *Cuphophyllus*) and *Cantharocybe* in the hygrophoroid clade has been indicated by Binder et al. (2010) and Ovrebo et al. (2011). The collections of *M. virosa* from India form the first report of a *Cantharocybe* species outside the North American continent.

Based on the results of the present molecular analysis, the following new taxonomic combination is proposed:

Cantharocybe virosa (Manim. & K.B. Vrinda) T.K.A. Kumar, **comb. nov.** Fig. 2
MycoBank MB800480

≡ *Megacollybia virosa* Manim. & K.B. Vrinda, Mycotaxon 111: 364. 2010.

Basidiomata medium-sized, fleshy, clitocyboid. Pileus 45–100 mm broad, convex, becoming broadly convex; surface light brown (6D5), brown (6E4), grayish brown (7E3), or dark gray (7F8), slightly darker at the center, pruinose to somewhat granular to the naked eye, with fine appressed scales under a lens, dry; margin inrolled when very young, becoming incurved and finally becoming straight, initially entire, becoming fissile with age. Lamellae adnate to decurrent, moderately crowded, with lamellulae in four to eight tiers, up to 9 mm deep, whitish to yellowish white (1A2); edges smooth to finely fimbriate under a lens. Stipe 20–75 × 5–23 mm, central or at times slightly excentric, terete to slightly compressed, almost equal with a dilated apex, solid, with white basal mycelium; surface dull white with fine, light brown (6D5), grayish

Figure 2. *Cantharocybe virosa* (TENN063485, holotype).
a. Basidiomata; **b**. Cheilocystidium; **c**. Basidiospore. Scale bars: a = 1 cm; b, c = 10 µm.

brown (7E3), or dark gray granular squamules concentrated towards the base and disappearing easily when handled. Context ≤20 mm thick, white. Odour strong and unpleasant. Spore print white.

Basidiospores 6.5–11(–12) × 5–7 µm, subglobose to ellipsoid, thin-walled, smooth, with refractive guttules, inamyloid. Basidia 23–56 × 7.5–11 µm, cylindrico-clavate to clavate, thin-walled, hyaline, with granular contents, 4-spored; sterigmata ≤5 µm long. Cheilocystidia abundant, 20–63 × 5.5–9 µm, clavate, cylindrico-clavate, or ventricose, majority with a small capitellum on a slender neck up to 35 µm long, often septate, thin-walled, hyaline to pale yellowish, apex sometimes covered with glutinous exudates; edges of lamellae sterile. Pleurocystidia absent. Lamellar trama regular to subregular, hyphae 3–15 µm wide, hyaline to pale yellowish, thin-walled, inamyloid. Pileal trama interwoven; hyphae 2–20 µm wide, slightly inflated, hyaline to pale yellowish, thin-walled. Pileipellis mostly a cutis of highly interwoven agglutinated hyphae, occasionally disrupted with trichodermal patches, hyphae 3–10 µm wide, thin- to slightly thick-walled, often with a grayish brown plasmatic pigment; terminal elements cystidioid, 23–85 × 3–10 µm, similar to cheilocystidia in all aspects, often with a greyish brown plasmatic pigment. Stipitipellis a highly disrupted and irregular cutis with agglutinated trichodermal patches of ascending to erect hyphal elements, hyphae 2–21 µm wide, thin- to slightly thick-walled, with gray to dark grayish plasmatic and membrane pigment; terminal elements cystidioid, similar to cheilocystidia in all aspects. Clamps frequent on all hyphae.

Habitat: On soil or on mud walls, often associated with roots of coconut trees in a tropical climate, solitary or in caespitose clusters, May to August.

Specimens examined — **India**, **Kerala State**, Calicut District, Puthiyangadi, 31 July 2005, T.K. Arun Kumar AK373 (TENN063483, GenBank, JX101471); 10 August 2005, T.K. Arun Kumar AK378 (TENN063476); 12 June. 2006, T.K. Arun Kumar AK395 (TENN063485, holotype); 21 June 2006, T.K. Arun Kumar AK397 (TENN063486); Thiruvananthapuram District, Plamood, 30 May 1998, C.K. Pradeep TBGT4310 (TENN063480); Vizhinjam, 11 December 2000, C.K. Pradeep TBGT5249 (TENN063479); 23 May 2002, C.K. Pradeep TBGT5526 (TENN063482); Muttada, 9 July 2006, K.B. Vrinda TBGT9804 (TENN063481); 28 August 2006, K.B. Vrinda TBGT9938 (TENN063484).

Key to the species of *Cantharocybe*

1a. Pileus dark brown; cheilocystidia resembling basidia with 1–4 sterigma-like apical appendages, extending at oblique angles and frequently swollen or capitate at the apex; basidiospores 9–9.5 × 5.5–6 µm, Belize, Central America (tropical) ..... ***C. brunneovelutina***

1b. Pileus yellow or pale grayish brown; cheilocystidia lecythiform or sometimes with a mucronate apex **2**

2a. Pileus yellow; basidiospores (13.5)14–15(16) × 3 5.5–6 µm, narrowly elliptic to oblong, western North America (temperate) . . . . . ***C. gruberi***

2b. Pileus pale grayish brown; basidiospores 6.5–11(–12) × 5–7 µm, subglobose to broadly ellipsoid, India (tropical) . . . . . ***C. virosa***

Cantharocybe virosa is known to be toxic, capable of inducing severe gastro-intestinal upset when eaten (Manimohan et al. 2010). Nothing is known about the potential toxicity of the two American species.

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